AS

POLYGLOT INTERNATIONAL
Global Management of Language-Related Projects

340 Brannan Street, Fifth Floor
San Francisco, CA 94107 • USA

Tel (415) 512-8800
FAX (415) 512-8982

TRANSLATION FROM GERMAN

PCT

INTERNATIONAL APPLICATION PUBLISHED IN ACCORDANCE WITH THE INTERNATIONAL PATENT COOPERATION TREATY (PCT)

(51) **International patent classification**[3]:
B01D 15/08; B01J 20/30; G01N 31/06 **A1**

(11) **International publication number: WO 83/03363**

(43) **International publication date:** October 13, 1983 (10.13.83)

---

(21) **International application number:** PCT/DE83/00056

(22) **International application date:** March 25, 1983 (03.25.83)

(31) **Priority application number:** P 32 11 309.9

(32) **Priority date:** March 26, 1982 (03.26.82)

(33) **Priority nation:** DE

(71)(72) **Applicants and inventors:** RIESNER, Detlev [DE/DE]; Eichenwand 15, D-4000 Düsseldorf 12 (DE). COLPLAN, Merin [TR/DE]; Christoph-Strasse 67, D-4000 Düsseldorf 1 (DE)

(74) **Attorneys:** HAFT, Uwe etc.; Hans-Sachs-Strasse 5, D-8000 Munich 5 (DE)

(81) **Designated treaty nations:** AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK, FR (European patent), GB (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US

Published

With an international search report.

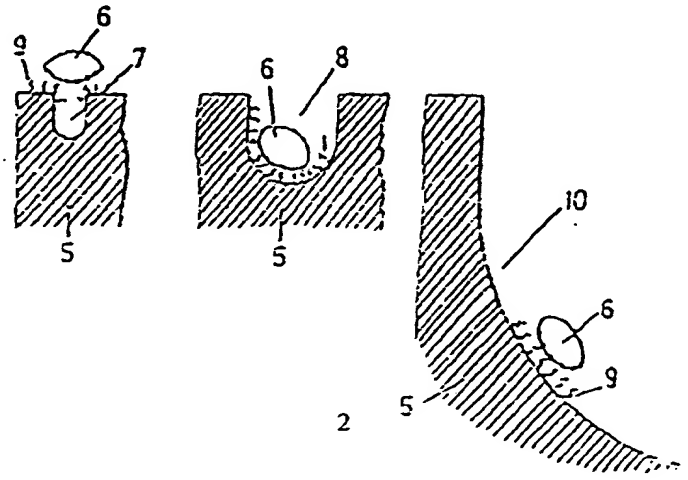
With modified claims.

---

(54) **Title:** CHROMATOGRAPHIC METHOD FOR ISOLATING MACROMOLECULES

(57) **Abstract:**

Various macromolecular compositions may be separated into their various components by means of a chromatographic method. The interactions of the macromolecules with the chromatographic carrier takes place in monodispersed size cavities with chemically modified surface. To solve a separation problem, a carrier material (5) is selected with cavities (7), (8) of which the diameter is correlated with the dimensions of the macromolecules (6) to be isolated and of which the chemical modification allows an optimum non-covalent interaction with the macromolecule. The interaction is further enhanced by attaching the interconnection groups by flexible chain molecules (9) to the carrier material and by excluding bivalent ions from all solvents.



Codes on the headers of specifications for the identification of PCT nations which publish international applications in accordance with the PCT.

AT	Austria
AU	Australia
BE	Belgium
BR	Brazil
CF	Central African Republic
CG	Congo
CH	Switzerland
CM	Cameroon
DE	Federal Republic of Germany
DK	Denmark
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
HU	Hungary
JP	Japan
KP	Peoples' Democratic Republic of Korea
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
MC	Monaco
MG	Madagascar
MR	Mauritania
MW	Malawi
NL	The Netherlands
NO	Norway
RO	Romania
SE	Sweden
SN	Senegal
SU	[the former] Soviet Union
TD	Chad
TG	Togo
US	United States of America

Chromatographic method for separating macromolecules

The invention pertains to a chromatographic process in which the carrier materials, which contain cavities, and the surface modification of the carrier materials have been optimized for special separating problems. The advances in biochemistry, molecular biology and polymer chemistry and their application in technology, medicine, pharmacy and genetic engineering require the rapid and systematic separation and isolation of macromolecules. Long chain oligonucleotides, high molecular nucleic acids and proteins are of special interest in the biological sciences in this connection. Thus in genetic engineering, for example, the problem frequently arises that a single molecular species has to be purified, to the point of complete homogeneity, from a naturally occurring mixture of 100 or more different high molecular nucleic acids. As is known, the individual nucleic acids can be characterized by their molecular weight, size and shape.

Chromatographic processes have proven to be advantageous for many separating problems. In this connection, high pressure liquid chromatography (HPLC) offers the greatest number of advantages in regard to resolution, low expenditure of time and reproducibility. This method was used previously in the form of gel permeation chromatography (GPC), ion exchange chromatography and reversed phase chromatography (RP chromatography). The following disadvantages arose in this way for the separating problems which are to be dealt with here.

GPC is capable of separating only very small molecules from very large ones.

Previously known ion exchangers and reversed phase carrier materials were capable of being used with high resolution only with small macromolecules such as oligonucleotides, peptides and protein fragments. The required resolution could not be attained for the separation of high molecular macromolecules such as, for example, messenger ribonucleic acids, viruses, viroids, deoxyribonucleic acid fragments and large proteins.

The hydrophobic/ionic chromatographic material RPC-5 such as, for example, that described by Larson, J.E. et al. (The Journal of Biological Chemistry (1979) 254, 5535-5541) was used with success for separating deoxyribonucleic acid fragments but it cannot be adapted to HPLC since the carrier material is not stable with respect to pressure. One therefore has to put up with low flow rates, i.e. very long chromatography times, and low chromatographic stability as disadvantages. It was not possible to separate complex ribonucleic acid mixtures from protein mixtures because of the chemical properties of the RPC-5 material.

Using previously known chromatographic processes, it was not possible, with high resolution and high speed, to separate complex macromolecular mixtures into their individual molecular species and to isolate them or analyze these mixtures.

The invention sets for itself the task of indicating a process and a device with which the aforementioned disadvantages are avoided. In particular, it is required that one be capable of separating -- in a single pass and with very high resolution and at a high rate of throughput -- macromolecular mixtures of the most widely differing types into their components, whereby the mixtures contain components with very different dimensions, e.g. in the range from 30 Å up to 1000 Å. In addition, the materials that are used should be capable of use at high pressure, over wide temperature ranges and with a long life. A high loadability with the macromolecular mixtures, that are to be separated, is desirable. This task is accomplished in accordance with the invention which is described in Claim 1 and the following claims. Thus, in contrast, to the current opinion in the technical literature, a chromatographic process has been developed and the carrier materials, which are necessary for this purpose, have been synthesized with which it is possible to separate -- with a very high resolving capacity -- complex macromolecular mixtures with a very wide spectrum of molecular sizes. Because of its simplicity and the inexpensive and stable materials as well as its economical application, this process is suitable for industrial usage in particular.

In contrast to previously known processes, which depended exclusively on the chemical properties of the carrier materials, the present invention proceeds on the basis of the knowledge that the size and/or shape of the cavities of the carrier materials are of quite essential significance for separation purposes and that they have to bear a certain relationship to the size of the macromolecular species that are to be isolated. It has been found that the size of the cavities has to amount to 1-20 times that of the component which is to be isolated. If the dimensions of the individual components, that are to be separated, differ from one another by more than a factor of 20, then it is, of course, possible to carry out separation in several steps.

A suitable modification of the surface is provided in accordance with an advantageous further development of the invention. In this connection, it has proven to be very advantageous if the groups, which are responsible for the interaction with the substances which are to be separated, are anchored onto the surface via flexible chain molecules. This action is achieved, for example, as a result of the use of g-glycidyloxypropyl-trimethoxysilane and N,N-dimethylaminoethanol. As far as the interacting groups are concerned, consideration can be given to strongly and weakly basic anion exchangers, strongly and weakly acidic cation exchangers, groups with hydrophobic interactions, groups with polarization interactions and groups which combine several of the designated properties.

Since, in the case of many applications, it has been found that bivalent metal ions can give rise to considerable interference, the proposal is also made in accordance with the invention that all parts, which come into contact with the solvents, should consist of a noble metal, plastic or glass or, as the case may be, have appropriate coatings.

The invention is elucidated in more detail on the basis of the figures.

The following aspects are shown.

Figure 1 shows a device, which is known as such, for the implementation of a chromatographic process.

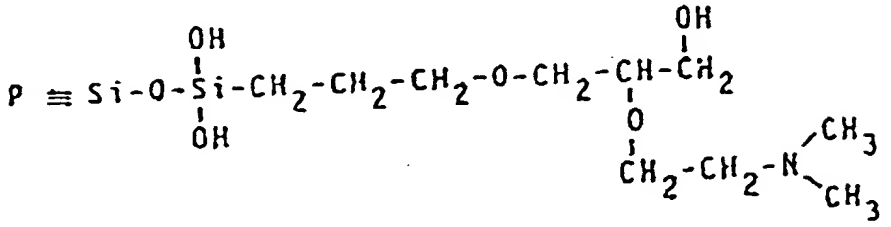
Figure 2 shows sections of cross sections through carrier materials with various sizes of cavities.

Figures 3, 4 and 5 show a graphic representation of various elution profiles for various sizes of cavities and sizes of molecules.

The device, which is illustrated in Figure 1, consists of a pressure-resistant cylinder (2) that is provided with a supply connection unit (1) and a run off connection unit (3). A very fine mesh, chemically inert sieve (4) is arranged in the lower part of the cylinder (2). The chromatographic carrier, that consists of discrete macro-porous silica gel particles (5), is located above the sieve (4). The substances, that are to be separated, are introduced in solution via the supply connection unit (1) and are absorbed in the cavities of the carrier (5). During the subsequent elution step, the substances and molecules, that are to be separated, are eluted via a solvent of continuously varying composition. Because of the change in the solvent over the course of time, the separated components emerge sequentially in the effluent (3) over the course of time.

The effect of the size of the cavities of the carrier material on the interaction with the macromolecule is elucidated on the basis of Figure 2. A macromolecule (6) cannot penetrate satisfactorily into an excessively small cavity (7) of the carrier material (5) in order to enter into optimum interaction. By contrast, the cavity (8), which is more favorable as a result of its dimensions, permits very intensive interactions. In order to increase the interaction, the interacting groups are anchored onto the surface of the cavity via flexible chain molecules (9). If, by contrast, the cavity (10) is too large, then one again has to reckon on a decrease in the interaction. The chain

molecules in this example have the following chemical structure:



The flexible chain g-glycidyloxypropyltrimethoxysilane is anchored on one side on the carrier silica gel (P) and carries the N,N-dimethylamino anion exchange group at its other end.

The chemical modification, that has been described, was used in spherical silica gels with a grain size of 10 mm and cavity sizes of 100 Å, 500 Å and 4000 Å. For this purpose, 50 g of silica gel particles are activated in a 1000 ml three-neck flask for 24 hours at a pressure of < 1 mbar and a temperature of 240°C. After cooling, ventilation took place with dry nitrogen and mixing took place with 500 ml of dry g-glycidyloxypropyltri-methoxysilane. The reaction took place for 8 hours at 220°C under a nitrogen atmosphere together with steady stirring. After the reaction, the excess g-glycidyloxypropyltrimethoxysilane is removed by means of suction and the epoxy-silica product is washed several times with dry dioxane. The epoxy silica was mixed with 500 ml of dry N,N-dimethylaminoethanol using a four-neck flask with an internal thermometer, a reflux condenser, a stirrer and a nitrogen inlet tube. The reaction was catalyzed by means of 1 ml of BF_3/ether and boiling took place under reflux for 24 hours. After the reaction, the dimethylamino silica gel was removed by suction and washed several times with dioxane, methanol and ether and then dried at 50°C. The yield amounted to 51.5 g.

Examples of the separation of short chain nucleic acids (Figure 3) and long chain nucleic acids (Figures 4 and 5) are illustrated in Figures 3 through 5. The diameters of the cavities are 100 Å, 500 Å and 4000 Å as indicated in the drawing. The concentration of the eluted components is measured via their UV absorption at 260 nm and is plotted against time.

The following results can be read off. Short chain nucleic acids with a length of 20 Å to 30 Å are separated optimally in a cavity size of 100 Å (Figure 3). As an example of the separation of long chain nucleic acids, we selected a natural mixture of transfer RNA (80 Å size), ribosomal 5SRNA (110 Å size), 9S messenger RNA (300 Å size) and viroid RNA (450 Å size, a plant-based pathogenic infectious RNA). It can be seen clearly from Figure 4 that the largest pore size, which was selected, results in the best separation, whereby the aspect is not ruled out that still better separation could have been achieved with a cavity size which lies between 500 Å and 4000 Å. The example from Figure 4 is further optimized in Figure 5 as a result of slower elution. Complete separation of all four components is achieved.

The dimethylamino silica gels, that were used in the examples that have been given, had a loadability of 4.8 mg of nucleic acid mixture/g (100 Å), 17.2 mg nucleic acid mixture/g (500 Å) and 5.6 mg of nucleic acid mixture/g (4000 Å).

The process, that has been described in accordance with the invention, is the first chromatographic process that has the following properties.

1. General applicability for the separation of macromolecules.

2. Short chromatography times and high reproducibility of the elution profiles as a result of the use of pressure-stable carriers in HPLC devices.

3. High loadability.

4. Chromatography materials with long-term stability (no "bleeding out" of the chromatography columns).

Patent Claims

1. Chromatographic process for the isolation of macromolecules by using carriers which contain pore-type cavities, characterized by the feature that use is made of a carrier, which contains pore-like cavities, in which the diameters of the cavities have 1-20 times the dimensions of the macromolecules and/or macromolecular aggregates that are to be separated.

2. Process in accordance with Claim 1, characterized by the feature that the size of the cavities is optimized in terms of the largest dimension in each case of the macromolecular species that is to be isolated.

3. Process in accordance with Claim 1, characterized by the feature that the size of the cavities is optimized in terms of the maximum dimensions of the macromolecular species that are contained in the mixture.

4. Process in accordance with Claims 1 through 3, characterized by the feature that the cross sections of the cavities are as constant as possible.

5. Process in accordance with Claims 1 through 3, characterized by the feature that the cross sections of the cavities can be a factor 3 larger or smaller than the optimum cross section.

6. Process in accordance with Claims 1 through 4, characterized by the feature that the cross section of the cavities is circular and the surface is hemispherical.

7. Process in accordance with Claims 1 through 4, characterized by the feature that the cavities are tubular.

8. Process in accordance with one or more of the previously designated claims, characterized by the feature that the solution, which contains the macromolecules which are to be separated, and the elution agents, are kept free from bivalent metal ions, e.g. as a result of the use of a noble metal, glass and/or plastic for the columns, tubes, valves or, as the case may be, pumps.

9. Device for the implementation of the process in accordance with one or more of the Claims 1 through 7, characterized by the feature that the surfaces of the cavities are modified in such a way that non-covalent interactions are ensured with the macromolecules that are to be separated.

10. Device in accordance with one or more of the previously designated claims, characterized by a modification of the surfaces of the hollow cavities that brings about a strongly or weakly basic anionic interaction.

11. Device in accordance with one or more of the previously designated claims, characterized by a modification of the surfaces of the hollow cavities that brings about a strongly or weakly acidic cationic interaction.

12. Device in accordance with one or more of the previously designated claims, characterized by a modification of the surfaces of the cavities which brings about a hydrophobic interaction.

13. Device in accordance with one or more of the previously designated claims, characterized by a modification of the surfaces of the cavities which brings about a polarization interaction.

14. Device in accordance with one or more of the previously designated claims, characterized by a combination of the interactions which are designated in Claims 9 through 13.

15. Device in accordance with one or more of the previously designated claims, characterized by the feature that the modification of the surfaces of the cavities takes place by means of a chemical substance which carries groups of flexible chain molecules which are responsible for the interaction.

16. Device in accordance with one or more of the previously designated claims, characterized by the feature that the carrier, which forms the cavities, consists of spherical or fragmented materials with a cavity size of 100 Å to 10,000 Å and a specific surface of 10 m^2/g to 800 m^2/g and, especially, between 500 Å to 4000 Å with a specific surface of 5 m^2/g to 200 m^2/g and a grain size, which is as monodisperse as possible, between 3 mm and 100 mm.

17. Device in accordance with one or more of the previously designated claims, characterized by the feature that the carriers, which have the cavities, can consist of silicon dioxide, whether this be amorphous or crystalline, aluminosilicates, aluminum oxides or carrier substances which have been coated with these materials.

18. Device for the [type of] preparation in accordance with Claims 9 through 17, characterized by the following steps:

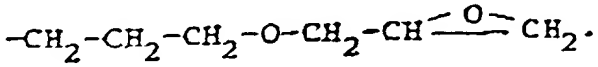
a) the carrier material which contains hollow cavities is reacted with a silanizing reagent of general formula

$$R_1R_2R_3Si\,R_4 \qquad I$$

R_1 corresponds to an alkoxy residue with 1 to 10 C-atoms, especially $-OCH_3$, $-OC_2H_5$ or $-OC_3H_7$ or a halogen atom, especially -Cl, or a dialkylamino group with identical or different alkyl residues with 1 to 6 C-atoms.

R_2 and R_3 correspond to a hydrocarbon residue with 1 to 10 C-atoms, especially -CH_3, -C_2H_5 or -C_3H_7 or an alkoxy residue with 1 to 10 C-atoms, especially -OCH_3, -OC_2H_5 or -OC_3H_7 or a halogen atom or an alkyl residue with 4 to 20 C-atoms, whereby the alkyl residue is interrupted by at least one oxa group or amino group and whereby this residue can also be substituted singly or multiply by a halogen group, a cyano group, a nitro group, an amino group, a monoalkylamino group, a dialkylamino group, a hydroxy group or an aryl group.

R_4 corresponds to a hydrocarbon chain with 1 to 20 C-atoms or to an alkyl residue, which is interrupted by at least one oxa group or amino group, whereby this residue can also be singly or multiply substituted with a halogen group, a cyano group, a nitro group, an amino group, a monoalkylamino group, a dialkylamino group, an alkoxy group, a hydroxy group, an aryl and/or an epoxy group, especially



b) In a second step, the carrier, that contains the cavities, is reacted with a reagent of general formula

X-R-Y II

to give the final chromatography material. X consists of an amino group, a hydroxy group, an epoxy group or a halogen atom.

R consists of a hydrocarbon chain with 2 to 20 C-atoms or an alkyl residue which has been interrupted by at least one oxa group or amino group, whereby this residue can also be substituted singly or multiply by a halogen group, a cyano group, a nitro

group, an amino group, a monoalkylamino group, a dialkylamino group, an alkoxy group, a hydroxy group, an aryl group and/or an epoxy group.

Y corresponds to a hydrocarbon residue with functional groups with 1 to 10 C-atoms, whereby the functional groups form anion exchangers or cation exchangers and which can be substituted singly or multiply by amino groups, monoalkylamino groups, dialkylamino groups, quaternary alkylamino groups, carboxyl groups, boric acid, alkylsulfonic acid and arylsulfonic acid.

MODIFIED CLAIMS

[received at the International Office on August 30, 1983 (08.30.83); the original Claim 1 is replaced by the new Claim 1 whose text follows. The original Claims 2 through 18 remain unchanged].

1. Chromatographic process for the isolation and analysis of macromolecules, especially nucleic acids and proteins, by using carriers which contain pore-type cavities, characterized by the feature that use is made of a carrier, which contains pore-like cavities, in which the diameters of the cavities have 1-20 times the dimensions of the macromolecules and/or macromolecular aggregates that are to be separated and the surfaces of the cavities are suitable for physical interaction with the macromolecules which are to be separated.

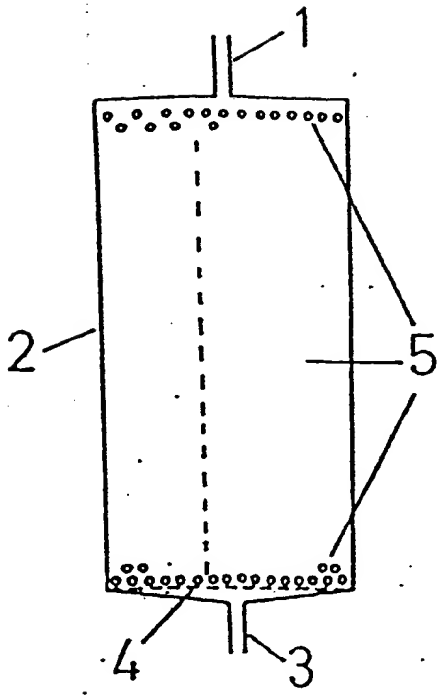


Fig.1

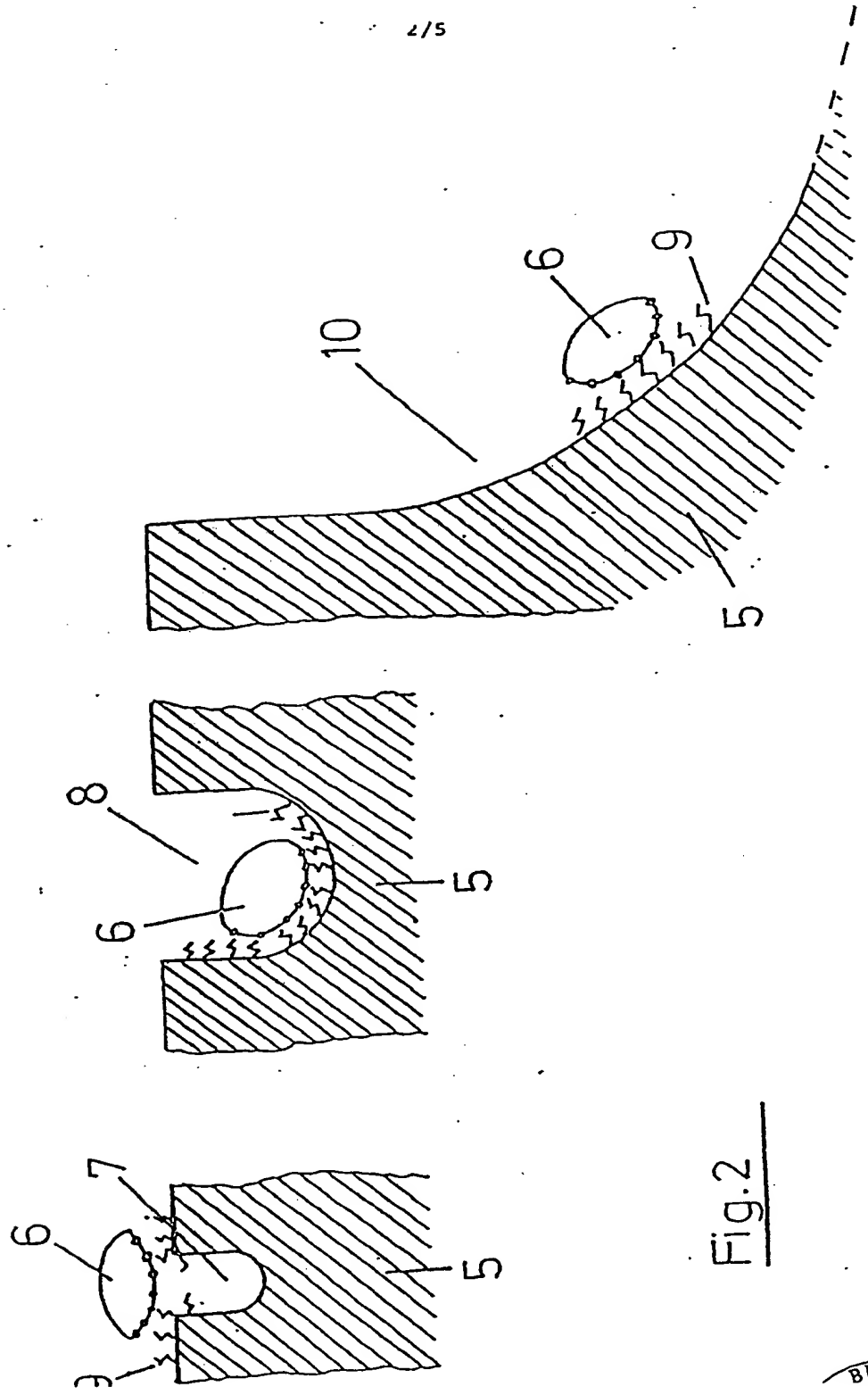


Fig.2

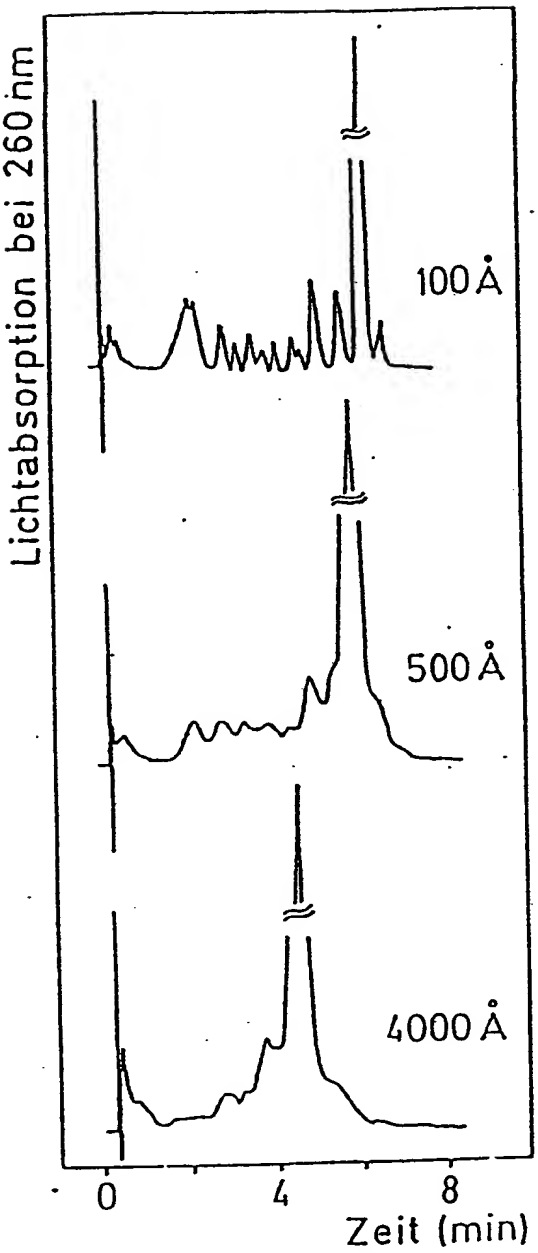
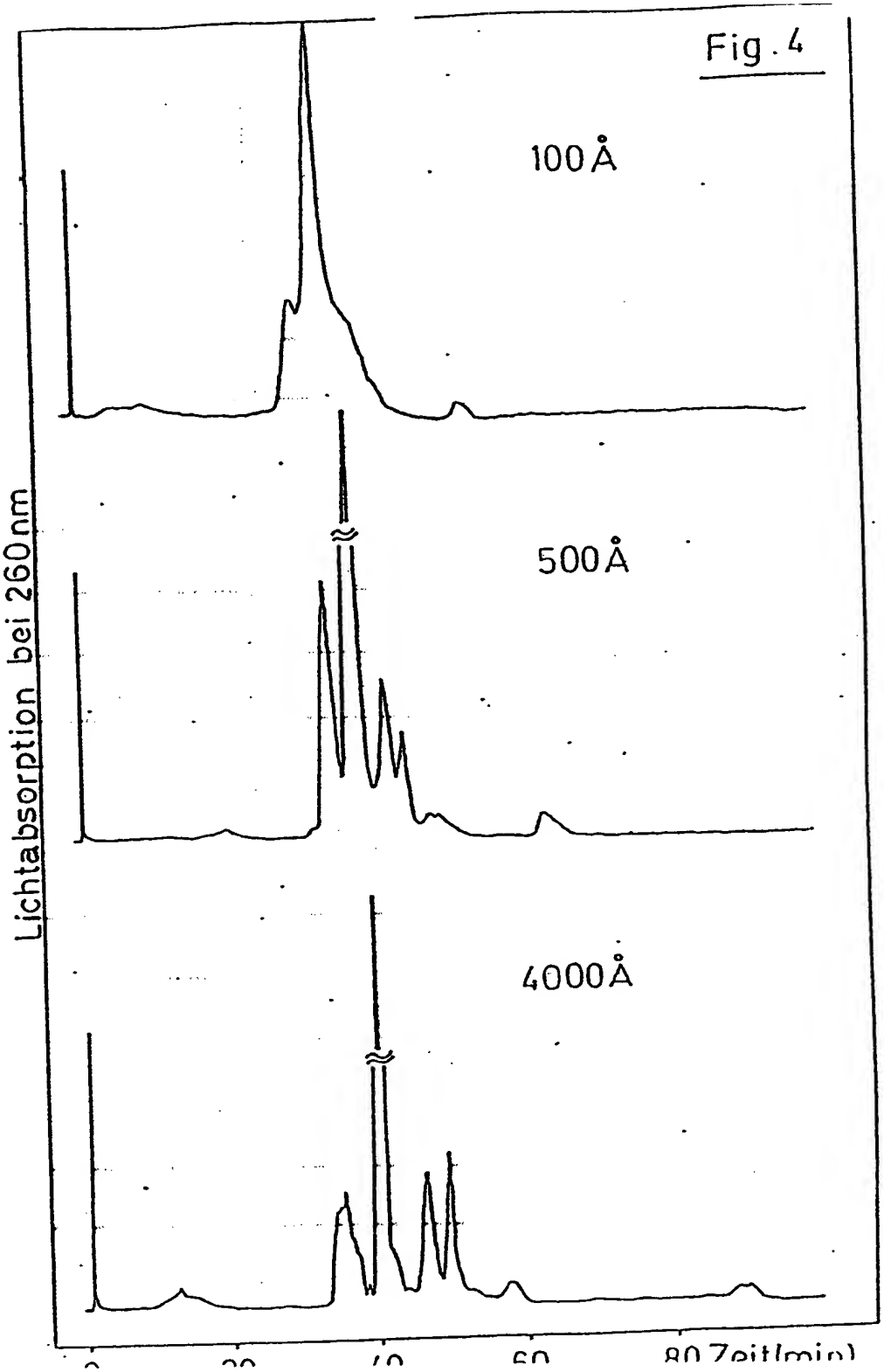


Fig. 3

Fig. 4



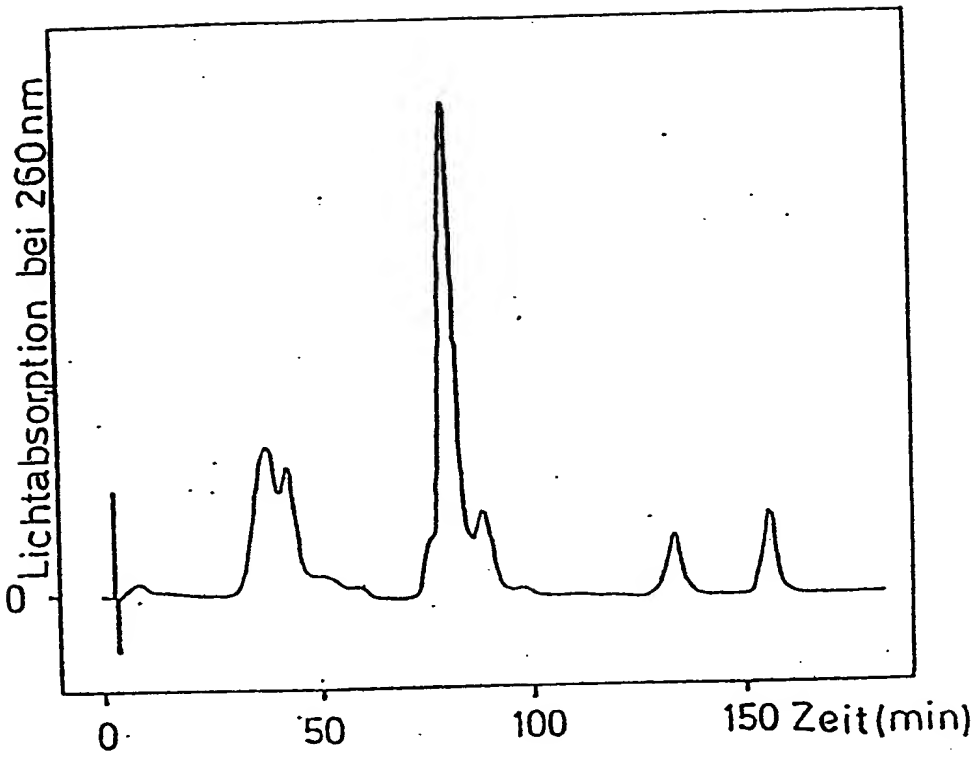


Fig.5

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.[3] B 01 D 15/08; B 01 J 20/30; G 01 N 31/06

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
Int.Cl.[3]	B 01 D; B 01 J

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category [6]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	DE, A, 2601930 (U.K.A.E.A.) 21 July 1977, see page 13, line 12 - page 19, line 22; pages 31-34, examples 9-10	1,2,9,16,17
Y	US, A, 4029583 (HO CHANG) 14 June 1977, see column 4, line 5 - column 16, line 47	1,9-18
Y	GB, A, 2075362 (KURARA.Y) 18 November 1981; see page 1, lines 42-53, line 62, page 4, example 1; page 7, example 8, page 10, claims 1-25	1-6,9-18
A	US, A, 4118316 (TALLEY) 03 October 1978, see columns 8-12; claims 1-17	1,9,10,16-18
A	US, A, 4140653 (IMURA) 20 February 1979, see columns 6-8; claims 1-9	1,9,11
A	FR, A, 2462183 (MERK PATENT) 13 February 1981, see pages 25-26; claims 1-3	1,9,12

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [3]	Date of Mailing of this International Search Report [3]
17 June 1983 (17.06.83)	15 July 1983 (15.07.83)
International Searching Authority [1]	**Signature of Authorized Officer [20]**
European Patent Office	

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 12/07/83

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
DE-A- 2601930	21/07/77	None		
US-A- 4029583	14/06/77	None		
GB-A- 2075362	18/11/81	FR-A-	2480606	23/10/81
		JP-A-	56147710	16/11/81
		DE-A-	3115608	18/03/82
		US-A-	4384954	24/05/83
		JP-A-	56147711	16/11/81
		JP-A-	57056038	03/04/82
		JP-A-	57056039	03/04/82
		JP-A-	57075141	11/05/82
US-A- 4118316	03/10/78	None		
US-A- 4140653	20/02/79	None		
FR-A- 2462183	13/02/81	DE-A-	2930516	12/02/81
		JP-A-	56021643	28/02/81

For more details about this annex :
see Official Journal of the European Patent Office, No. 12/82

Internationales Aktenzeichen PCT/DE 83/00056

I. KLASSIFIKATION DES ANMELDUNGSGEGENSTANDS (bei mehreren Klassifikationssymbolen sind alle anzugeben)*

Nach der Internationalen Patentklassifikation (IPC) oder nach der nationalen Klassifikation und der IPC

Int.Kl.³ B 01 D 15/08; B 01 J 20/30; G 01 N 31/06

II. RECHERCHIERTE SACHGEBIETE

Recherchierter Mindestprüfstoff*

Klassifikationssystem	Klassifikationssymbole
Int.Kl.³	B 01 D; B 01 J

Recherchierte nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Sachgebiete fallen⁵

III. EINSCHLÄGIGE VERÖFFENTLICHUNGEN¹⁰

Art*	Kennzeichnung der Veröffentlichung, soweit erforderlich unter Angabe der Maßgeblichen Teile¹²	Betr. Anspruch Nr.¹³
X	DE, A, 2601930 (U.K.A.E.A.) 21. Juli 1977, siehe Seite 13, Zeile 12 - Seite 19, Zeile 22; Seiten 31-34, Beispiele 9-10 --	1,2,9,16, 17
Y	US, A, 4029583 (HO-CHANG) 14. Juni 1977, siehe Spalte 4, Zeile 5 - Spalte 16, Zeile 47 --	1,9-18
Y	GB, A, 2075362 (KURARAY) 18. November 1981, siehe Seite 1, Zeilen 42-53, Zeile 62; Seite 4, Beispiel 1; Seite 7, Beispiel 8; Seite 10, Ansprüche 1-25 --	1-6,9-18
A	US, A, 4118316 (TALLEY) 3. Oktober 1978, siehe Spalten 8-12; Ansprüche 1-17 --	1,9,10, 16-18
A	US, A, 4140653 (IMURA) 20. Februar 1979, siehe Spalten 6-8; Ansprüche 1-9 --	1,9,11

./.

* Besondere Kategorien von angegebenen Veröffentlichungen¹⁴:

"A" Veröffentlichung, die den allgemeinen Stand der Technik definiert, aber nicht als besonders bedeutsam anzusehen ist

"E" älteres Dokument, das jedoch erst am oder nach dem internationalen Anmeldedatum veröffentlicht worden ist

"L" Veröffentlichung, die geeignet ist, einen Prioritätsanspruch zweifelhaft erscheinen zu lassen, oder durch die das Veröffentlichungsdatum einer anderen im Recherchenbericht genannten Veröffentlichung belegt werden soll oder die aus einem anderen besonderen Grund angegeben ist (wie ausgeführt)

"O" Veröffentlichung, die sich auf eine mündliche Offenbarung, eine Benutzung, eine Ausstellung oder andere Maßnahmen bezieht

"P" Veröffentlichung, die vor dem internationalen Anmeldedatum, aber nach dem beanspruchten Prioritätsdatum veröffentlicht worden ist

"T" Spätere Veröffentlichung, die nach dem internationalen Anmeldedatum oder dem Prioritätsdatum veröffentlicht worden ist und mit der Anmeldung nicht kollidiert, sondern nur zum Verständnis des der Erfindung zugrundeliegenden Prinzips oder der ihr zugrundeliegenden Theorie angegeben ist

"X" Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann nicht als neu oder auf erfinderischer Tätigkeit beruhend betrachtet werden

"Y" Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann nicht als auf erfinderischer Tätigkeit beruhend betrachtet werden, wenn die Veröffentlichung mit einer oder mehreren anderen Veröffentlichungen dieser Kategorie in Verbindung gebracht wird und diese Verbindung für einen Fachmann naheliegend ist

"&" Veröffentlichung, die Mitglied derselben Patentfamilie ist

IV. BESCHEINIGUNG

Datum des Abschlusses der internationalen Recherche³	Absendedatum des internationalen Recherchenberichts³
17. Juni 1983	15 JUL 1983
Internationale Recherchenbehörde	**Unterschrift des bevollmächtigten Bediensteten²**
Europäisches Patentamt	G.L.M. Kruydenberg

III. EINSCHLAGIGE VEROFFENTLICHUNGEN (FORTSETZUNG VON BLATT 2)

Art*	Kennzeichnung der Veröffentlichung, ** soweit erforderlich unter Angabe der maßgeblichen Teile **	Betr. Anspruch Nr *
A	FR, A, 2462183 (MERK PATENT) 13. Februar 1981, siehe Seiten 25-26; Ansprüche 1-3	1,9,12

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Die Angaben über die Familienmitglieder entsprechen dem Stand der Datei des Europäischen Patentamts am 12/07/83

Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

Im Recherchenbericht angeführtes Patentdokument	Datum der Veröffentlichung	Mitglied(er) der Patentfamilie		Datum der Veröffentlichung
DE-A- 2601930	21/07/77	Keine		
US-A- 4029583	14/06/77	Keine		
GB-A- 2075362	18/11/81	FR-A-	2480606	23/10/81
		JP-A-	56147710	16/11/81
		DE-A-	3115608	18/03/82
		US-A-	4384954	24/05/83
		JP-A-	56147711	16/11/81
		JP-A-	57056038	03/04/82
		JP-A-	57056039	03/04/82
		JP-A-	57075141	11/05/82
US-A- 4118316	03/10/78	Keine		
US-A- 4140653	20/02/79	Keine		
FR-A- 2462183	13/02/81	DE-A-	2930516	12/02/81
		JP-A-	56021643	28/02/81

Für nähere Einzelheiten zu diesem Anhang :
siehe Amtsblatt des Europäischen Patentamts, Nr. 12/82